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DEVELOPMENT OF SAMPLE PREPARATION METHODS FOR ANALYSIS OF MARINE ORGANISMS

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16. Abstract
A two-year laboratory investigation has been completed to develop laboratory methods for processing, extracting, purifying, concentrating, and measuring specific organic pollutants found in marine organisms. Major conclusions are as follows: a. Quantitative measurement of many organic contaminants is possible in the range of 0.2 to 0.5 parts per million in a 5-g sample. b. Qualitative detection is possible at concentrations below the limit of quantitative measurement, thus providing a means of identifying the presence of organic contaminants at levels far below any known threshold of toxicity or other adverse effects for most organic compounds. c. Compounds tested in laboratory studies included saturated hydrocarbons to C_{22}, aromatics to C_9, alcohols to C_7, amines to C_6, glycols to C_6, unsaturated hydrocarbons to C_{10}, as well as various ketones, phenols, esters, heterocyclic compounds, acids, sulfides, amides, and chlorinated hydrocarbons. With most of these, recovery of 70 to 90 percent of the amount present was obtained, indicating that quantitative measurements are possible within the ranges stated above. d. Several methods of sample preparation can be used prior to analysis by gas chromatography. e. Based on the results obtained, the analysis of almost any variety of marine specimens should be possible to measure trace organic constituents. (Hegre - EPA)

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SECTION I

SUMMARY AND CONCLUSIONS

A two-year laboratory investigation has been completed to develop laboratory methods for processing, extracting, purifying, concentrating, and measuring specific organic pollutants found in marine organisms. These methods provide new techniques for measuring organic contaminants in water to establish monitoring procedures, identify sources of contamination, evaluate methods of treatment, or for other uses in water quality management. Since individual chemical compounds can be measured, these methods are more specific than the conventional water quality parameters such as BOD, COD, etc.

Major conclusions are as follows:

- Quantitative measurement of many organic contaminants is possible in the range of 0.2 to 0.5 part per million in a 5-g sample. This limit of detection could be extended by increasing the sample size.

- Qualitative detection is possible at concentrations below the limit of quantitative measurement, thus providing a means of identifying the presence of organic contaminants at levels far below any known threshold of toxicity or other adverse effects for most organic compounds.

- Compounds tested in laboratory studies included saturated hydrocarbons to C_{22}, aromatics to C_9, alcohols to C_7, amines to C_6, glycols to C_6, unsaturated hydrocarbons to C_{10}, as well as various

ketones, phenols, esters, heterocyclic compounds, acids, sulfides, amides, and chlorinated hydrocarbons. With most of these, recovery of 70 to 90 percent of the amount present was obtained, indicating that quantitative measurements are possible within the ranges stated above.

- Several methods of sample preparation can be used prior to analysis by gas chromatography. The combination of methods which provides the greatest flexibility appears to be an extraction procedure with carbon tetrachloride, followed by a cleanup procedure using column chromatography to separate the organic contaminants being measured from naturally occurring oils and other interfering substances. Most of the classes of compounds investigated can be measured with a single sample using this combination of techniques. The greatest difficulties were encountered in measuring amines and glycols, and variations in extraction and cleanup procedure are necessary if best results are to be obtained with these two classes of compounds.

- Similar methods of sample preparation could be used with specimens of shrimp, oysters, or fish. Based on the results obtained, the analysis of almost any variety of marine specimens should be possible to measure trace organic constituents.

SECTION II
RECOMMENDATIONS

Since this report outlines the development of new methods of measurement, final recommendations for further action are not presented. However, the results obtained to date indicate profitable avenues for further investigation which are now being pursued. In addition, several practical applications of these methods of measurement are possible, and these are outlined briefly to indicate valuable future uses for this work.

A major advantage of this method of measurement is that it is based on the analysis of fish, shrimp, or other marine organisms collected in a bay or estuary, rather than on the direct analysis of water samples. This avoids many of the errors inherent in the sampling procedure since it is always difficult to be sure that a water sample is representative of the body of water being studied over some known period of time. However, marine organisms act as cumulative sampling devices, storing certain organic contaminants so that they can be measured by subsequent laboratory analysis. While some compounds may be decomposed or otherwise eliminated, many are accumulated and stored over an extended period of time and thus can be measured by chemical analysis.

In addition to the advantages inherent in this method of sampling, results are also obtained on individual organic compounds which are present in the sample. Therefore, the information obtained should be useful in many ways since this type of information is much more specific

than that obtained by using the conventional water quality parameters such as BOD, COD, DO, etc., to characterize waste streams or bodies of water. Possible applications include the following:

1. Water quality monitoring networks in bays and estuaries could be based at least in part on the analysis of marine organisms. Methods based on analysis of water samples are difficult to use to establish long-term patterns because of the combined effects of tide and wind action on mixing, and the resulting effects on the distribution of pollutants throughout the estuary.

2. Measurement of the direct effect of pollution on marine life should make it possible to decrease the number of sampling stations or the frequency of sampling. In addition, individual components responsible for taste and odor problems in fish and other marine organisms could be identified and measured.

3. Individual pollutants could be traced back to their source, in order to identify various sources that cause unusual adverse effects on the receiving body of water. This is especially important if a body of water receives waste effluents from a large industrial complex which includes many different types of operations.

4. Different methods of waste treatment could be evaluated to determine what treatment techniques can satisfactorily eliminate individual compounds found to be toxic, odorous, or otherwise objectionable.

SECTION III
INTRODUCTION

Objective

The overall objective of this program was to develop methods of sample preparation suitable for processing, extracting, purifying, and concentrating specific organic pollutants found in marine organisms, so that chemical analysis to measure these contaminants can be carried out. These methods in turn can be useful in the development and application of new techniques for water quality monitoring based on the analysis of marine organisms.

This investigation was directed primarily at the measurement of contaminants of industrial origin. Serious pollution problems exist from this type of contamination, and previous work has not been adequate to provide suitable methods of evaluating the effects of pollution on marine resources or on human health.

Plan of Operation

Shrimp, oysters, and fish were used to obtain representative samples of different types of organisms. These varieties occur commonly in Galveston Bay and other estuaries along the Texas coast and represent different degrees of mobility in that fish move readily, shrimp move more slowly, while oysters are immobile throughout most of their life cycle. This difference in mobility is important in view of the effects of tidal action on pollution in bays and estuaries.

These three species of marine organisms were obtained from various parts of Galveston Bay, particularly where high levels of contamination are most likely to be present, as shown in Figure 1.* Personnel of the Texas Parks and Wildlife Laboratory in Seabrook, Texas, have graciously given their assistance in this phase. Other species could also have been used, but it was felt that these varieties represented a logical choice for this study.

The general plan for the study involved obtaining specimens from a relatively polluted portion of Galveston Bay, for laboratory study to evaluate sample preparation methods. Spiked samples were used for much of the work in order to obtain quantitative results which would indicate the degree of recovery of the various contaminants. The specimens were treated to separate the organic contaminants prior to actual analysis. The selection of specific techniques depended on the nature of the contaminants which were measured and on their vapor pressure, chemical polarity, and other characteristics. In general, the various techniques which were used in the study fell into the following major categories:

- Sample preparation and cleanup methods.
- Solution in water or other suitable solvents to remove materials to be measured.

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*Tables and Figures are placed at the end of this report.

- Extraction with organic solvents to concentrate the contaminants in a smaller volume for subsequent analysis.
- Stripping with air or nitrogen to remove volatile materials from water or other substrate and thus provide a preliminary separation.
- Column chromatography, frequently useful to separate a mixture of trace organic materials from water and other constituents so that the organic fraction can be further analyzed.
- Gas chromatography, which provides a sensitive method for the analysis of the organic fraction.

Various combinations of these methods were used; in particular, extraction, column chromatography, and gas chromatography was the most useful combination. Various portions of the investigation devoted to the development of these techniques will be discussed separately later in this report.

SECTION IV
STUDY PROCEDURE

Literature Survey

A literature survey was conducted by reviewing available literature, especially for the last five years. Some selectivity was exercised to include only those analytical procedures which appeared to be most useful in the project. Because of the large volume of literature published on organic contaminants in water and marine organisms, only articles which covered the preparation, separation, and identification of these contaminants were investigated. The Bibliography is included in this report, to indicate available sources of information pertinent to the work performed. (See Appendix)

Development of Analytical Procedures

It was decided to concentrate primarily on gas chromatographic techniques for the analysis of organic contaminants. With the present technology on gas chromatography, numerous classes of organic compounds can now be analyzed, thus providing a broad capability with only limited use of other analytical techniques.

A gas chromatograph, especially one equipped with a flame ionization detector, is sensitive to extremely small amounts of organic compounds. Measurement of nanogram quantities of many organic constituents is routine for trace analysis applications.

The first step in the development of gas chromatographic techniques was to obtain an adequate reference library of chromatograms of different organic compounds that are most likely to be present as industrial contaminants in water. After some literature search, and based on previous experience, it was decided to use a number of columns which would perform the desired function. The columns selected were:

- 12 ft x 1/8 in. OD, 10% Apiezon L packed on Chromosorb P, 60/80 mesh
- 50 ft x 0.02 in. ID, silicone DC 550 open tubular column
- 100 ft x 0.02 in. ID, silicone DC 550 open tubular column
- 5 ft x 1/8 in. OD, silicone DC 550 packed on Chromosorb P, 60/80 mesh
- 6 ft x 1/8 in. OD, 20% SE 30 packed on Chromosorb P, 60/80 mesh
- 5 ft x 1/4 in. OD, 15% silicone DC 550 coated on 12.5% potassium hydroxide-treated Chromosorb P, 60/80 mesh
- 5 ft x 1/4 in. OD, 5% Reoplex 400 coated on acid-washed G-Chromosorb, 40/60 mesh.

The last two chromatographic columns were especially developed and prepared during this study for the detection and analysis of glycols and amines, respectively. These columns will be discussed fully under "Preparation of New Chromatographic Columns".

These columns were installed in a Perkin-Elmer 900 dual flame chromatograph instrument which was used for analysis throughout the investigation.

In addition to obtaining or developing these columns, some work was done to install a backflush system in the instrument. Such a system would be useful when injecting solvent-extracted samples into the chromatograph. It could provide a tool for backflushing heavy sample oils that might be extracted with the solvent and are not pertinent to the analysis and, also, might damage the columns. However, difficulties developed and the system was discontinued. It became unnecessary when a preliminary cleaning step was utilized prior to analysis.

Sample Preparation

At the beginning of the investigation, work on sample preparation was limited to oyster samples. Subsequently, shrimp and fish samples were used in modifying the methods so that any of the three could be used.

Oyster Sample Preparation. Several series of tests were conducted to evaluate different methods and procedures for grinding, extracting, and otherwise processing samples. From this experience, the following procedure was developed and used for subsequent experiments.

Fresh oyster samples collected at the same source were removed from their shells and composited in a glass container. Usually, these composite samples from each source were frozen unless they were

used immediately in laboratory experiments. The composite sample, which contained excess water, was transferred to a cheesecloth filter which was supported on a 500-ml beaker. The excess water was allowed to drain without exerting any pressure on the oyster specimen. The draining procedure was continued until no more liquid drained. The semi-dry oysters were chilled for one hour and then placed in a Waring blender and homogenized for about 30 seconds. The tissue slurry was poured into a glass container, covered, and refrigerated until further use. The sample was kept cold at all times to avoid losing low boiling organic constituents.

Prior to solvent extraction, a 5-g aliquot of the cold sample slurry was "spiked" by thoroughly mixing with a known weight of the organic contaminants to be investigated. In most of the experiments performed, the oyster sample slurry was spiked with 2.5 μg of each of the components studied. On a 5-g sample, this represented 0.5 ppm by weight for each component. The noise level of the chromatographic instrument is such that the limit of detection for many organic compounds is around 0.1 ppm for a 5-g sample. This limit could be extended through the use of larger samples.

Following the spiking, 10 g of anhydrous sodium sulfate (Na_2SO_4) was added to the mixture and thoroughly mixed. The purpose of the sodium sulfate was to absorb any water present which might lead to emulsion formation during the extraction procedure.

The final material was then refrigerated for two to three hours before extraction. Sometimes an unspiked sample was prepared simultaneously in the same manner to serve not only as a control but also to determine if any of the contaminants which were added to the spiked sample were present initially.

Shrimp Sample Preparation. The methods which were used in the preparation of shrimp samples were generally similar to those developed during the oyster study with some slight modifications.

On receipt of the shrimp samples, tissues were removed from the shells and composited in glass jars. The heads and tails were discarded. The procedures of draining the excess water, chilling, grinding, and homogenizing the shrimp tissues were the same procedures used in oyster sample preparation. However, the time required to grind the shrimp sample was about twice that needed for homogenizing oyster samples. This appeared to be the main difference between oyster and shrimp sample preparation.

Spiking an aliquot shrimp sample slurry with a known weight of the organic contaminants to be investigated and adding the 10 g of anhydrous sodium sulfate were carried out by the same procedures used in the oyster sample preparation.

In the series of experiments performed, the shrimp slurry was spiked with 2.5 μg of each of the components studied. On a 5-g

sample, this also represented 0.5 ppm by weight for each component. On certain occasions, a 5-g sample was spiked with 1.2 μg of components, as was the case with o-ethyl phenol, to obtain a concentration of approximately 0.25 ppm by weight.

Fish Sample Preparation. After developing satisfactory methods for the preparation of shrimp and oyster samples, further laboratory experience indicated that the same procedure was suitable for fish specimens. Most of the laboratory work was performed with sheepshead minnows averaging two grams in weight. Specimens were obtained from Galveston Bay near Seabrook, Texas, together with bay water used in laboratory experiments and in maintaining live specimens for later use. Salinity of the water used was about 16 ppt.

To prepare samples for analysis, tails and fins were removed and discarded after which the remaining material was drained of excess water, chilled, ground, and homogenized in the same manner as in the treatment of shrimp and oyster samples. Ground samples were then spiked with a known weight of the organic contaminants to be investigated, and anhydrous sodium sulfate was used to avoid the effects of excess water in the same manner as in the previous laboratory experiments. Through spiking of samples and comparing the analytical results with unspiked samples and control samples, it was found that the sensitivity and percent recovery with most organic contaminants was similar to that obtained

with shrimp and oyster samples. On a 5-g sample, measurement of various organic compounds in the fractional part per million concentration range was ccomplished routinely.

Grinding With Dry Ice. As a possible alternate procedure, laboratory experiments were conducted to evaluate the possibility of grinding specimens with dry ice while frozen. Dry ice has the advantage of subliming directly to gaseous carbon dioxide, thereby avoiding the problem of draining or otherwise disposing of water with the risk of losing a portion of the organic contaminants being measured. In all experiments, however, the water present in the original specimen proved troublesome, and it was not possible to keep the sample sufficiently frozen while grinding to avoid some drainage of liquid water. If a large amount of dry ice was used, this caused the water to freeze but did not avoid the subsequent drainage which occurred when the sample was thawed. Therefore, it appeared that the alternate procedure of tying up water with sodium sulfate followed by extraction with an organic solvent was a better method of avoiding emulsification and other problems inherent in the disposition of water, and the dry ice grinding procedure was abandoned.

Separation Procedures

Techniques used for the removal of organic contaminants from the prepared samples included distillation, organic solvent extraction, and water extraction. Each of these methods proved useful under certain circumstances, as discussed below.

Distillation Procedures. The distillation of micro amounts of organic compounds in the 200° - 300° C boiling point range can be successfully accomplished at low temperatures and at atmospheric pressure by using a purge gas such as nitrogen as a carrier. Consequently, extensive preliminary work was conducted in developing a micro distillation apparatus with the associated recovery traps that would perform successfully. Various existing designs of apparatus including distillation flasks and traps were investigated. A laboratory-prepared water standard containing benzene, toluene, ethyl benzene, paraxylene, meta-xylene, ortho-xylene, and cumene was used in the evaluation of the distillation system. The objective was to recover the distilled aromatic compounds, which were in the 1-μg range, by the best possible means. Various designs of traps, including U tubes, straight tubes, micro impingers, etc., were investigated. The cooling media included ice-salt and dry ice-acetone baths. Numerous distillations were performed at 100° C with no success in recovering the aromatic compounds.

Difficulty was encountered with the traps using dry ice-acetone coolant due to freezing and plugging. To overcome this problem, it was necessary to go to a large trap which of course is undesirable in micro analytical work. Consideration was then given to employing an absorbing solution to trap the aromatic distillate. Since it is not possible to absorb these compounds chemically as is the case with organic acids, amines,

and other reactive compounds, a solvent absorption step was considered. The solvent selected was carbon tetrachloride (CCl_4), which offered numerous advantages over other solvents, particularly in that it is heavier than water and thus will remain in the lower layer of the trap as the water distillate condenses.

Eventually, a micro distillation apparatus was designed as shown in Figure 2. This device consists of a 125-ml round bottom distillation flask and a series of micro traps connected together with a 1-mm ID Teflon tube. The traps were made from 6-mm ID Y-glass connectors which were sealed at the bottom to provide a small reservoir in which to place the solvent. To prevent thermal cracking of tissue samples, the distillation flask was totally immersed in a liquid bath rather than using a heating mantle, which usually produces hot spots around the flask. A hot liquid bath also provides better temperature control than other means. The nitrogen purge gas line was connected to the distillation flask, and a flow meter was attached to the outlet of the trap. This flow device is necessary to control the carrier gas flow through the system.

Distillations were performed on the aromatic standards using spiked oyster samples. It was established that at certain operating conditions, such as proper temperatures and nitrogen flow, aromatic compounds in the 1-μg range could be successfully recovered in a trap

containing 0.25 ml of carbon tetrachloride which was immersed in an ice-salt bath. Under the proper distillation conditions, which were established at a nitrogen flow of 10 cc per minute and distillation programming of from 28° to 100° C in about 15 minutes, very little loss of solvent from the trap occurred. The technique proved highly successful, yielding excellent recovery of the aromatic components as determined by gas chromatography. This technique will be discussed further in the discussion concerning aromatic compounds in the Appendix.

Organic Solvent Procedures. Another method for extracting and collecting organic contaminants from marine organisms prior to analysis was extraction with organic solvents.

Some of the organic solvents used for extracting contaminants from oyster and shrimp samples were n-hexane, n-dodecane, acetone, isopropyl ether, carbon tetrachloride, dioxane, chloroform, benzene, and ethyl alcohol. It was found that carbon tetrachloride offered more advantages than the other solvents for the compounds investigated in the study, because it elutes rapidly from the columns and gives sharp peaks with little tailing.

The extraction experiments involved the use of 100-ml centrifuge tubes. Five grams of the prepared ground tissue were placed in the centrifuge tube and extracted with a volume of 25 ml of the organic solvent. To provide better phase separation, sodium sulfate crystals

were added to the mixture during the extraction. The tube was then stoppered with a saran-wrapped rubber stopper and the sample shaken for one minute, then centrifuged at about 1500 rpm for three minutes. The solvent extract was then poured into a 100-ml test tube. The tissue mixture was further extracted with an additional 10 ml of carbon tetrachloride and the extract combined with the previous one. The test tube was then stoppered and refrigerated for further use.

Sometimes unspiked samples were also extracted simultaneously with the spiked samples for control purposes. The extraction experiments employing these procedures have shown that organic pollutants absorbed and retained by marine organisms can be extracted and analyzed in micro quantities.

Water Solvent Procedures. The extraction procedure using water as a solvent was identical to the organic solvent extraction. As an extraction solvent, water offered some advantages over carbon tetrachloride since it is a better solvent for certain organic contaminants such as glycols. However, the possibility of forming emulsions with water and naturally occurring organic constituents, and the difficulty in cleaning up the extracts, made carbon tetrachloride more advantageous to use. After a few experiments, the use of water as an extraction solvent was discontinued.

Cleanup and Concentration of Tissue Solvent Extract

The solvent extract obtained from oyster, shrimp, or fish tissues contained not only the added contaminants but also numerous naturally occurring oils and different dyes of unknown composition. The presence of these high molecular weight materials made the sample extracts after concentration undesirable for gas chromatographic analysis. Therefore, a cleanup procedure which would remove the foreign materials without removing the organic contaminants was essential at this point.

Description of "Cleanup" Column. Cleanup of the extracts by adsorption of the heavy oils and dyes on a solid adsorbent was carried out efficiently by liquid column chromatography. The column used was a 0.5-cm ID glass tube, 25-30 cm in length and tapered at the outlet to provide an orifice of 3 mm ID. This column was packed with Florosil (60/100 mesh) adsorbent and anhydrous sodium sulfate as illustrated in Figure 3. Such a column was good for cleaning up carbon tetrachloride tissue extracts. However, for best use with other solvents such as ethyl alcohol and dioxane, the length, diameter, and amount of Florosil would need to be changed for best results. When water was used as a solvent, there was no need to add anhydrous sodium sulfate to the column.

Other adsorbents such as magnesium carbonate plus silica gel 1:1, silica gel alone, alumina, silica gel plus alumina 1:1, and magnesium oxide plus celite 1:1 were tested for the removal of dyes

and oils in the tissue extracts. However, none of these adsorbents was found to be as good as Florosil in providing the degree of separation required.

The column was prewetted with the solvent, such as carbon tetrachloride, and the tissue extract was eluted through the column into a 50-ml beaker at a rate of 1/2 ml per minute. The elution was allowed to proceed without applying any pressure to the top of the column. An additional 5 ml of CCl_4 was added to the column after the tissue extract had passed through the column. It was found that this volume of CCl_4 was sufficient to elute all the components under study.

Solvent Evaporation Technique. The beaker containing the eluate was then placed in a water bath and kept at atmospheric pressure and a temperature approximately equal to the boiling point of the solvent (77° C for CCl_4). The sample was evaporated nearly to 0.25-ml volume, and then removed and cooled. Additional CCl_4 was added to the beaker, which was then shaken and the contents transferred to a graduated micro test tube. The contents of the tube were further subjected to evaporation in a water bath until the final volume was 0.25 ml, stoppered with a rubber septum, and set aside for investigation. This final sample, containing a known volume of solvent and the residue, was used in the chromatographic analysis

In the evaporation, it is usually necessary to adjust the solvent to an accurately known volume so that an aliquot can be taken for

the analytical step. Care must be taken that the solvent is not lost by evaporation when the residue is made up to a standard volume and an aliquot is withdrawn for analysis.

Analysis by Gas-Liquid Chromatography

Specifications for the Gas Chromatographic System Used. Chromatography is one of the most useful techniques for organic compounds available to the analytical chemist. By this method, complex organic mixtures of compounds can be separated and the individual components detected and sometimes identified.

The instrument employed in this study was a Perkin-Elmer 900 gas chromatograph with a dual ionization detector. This instrument is extremely sensitive to micro amounts of organic compounds. It is also fast, and gives good resolution of complex mixtures.

Almost all the organic compounds used in the experiments were analytical standards obtained from Poly Science Corporation Chemical Division, Evanston, Illinois.

Preparation of New Chromatographic Columns. Amines and glycols were the most troublesome classes of organic compounds investigated, from the standpoint of analysis by gas chromatography. This was not surprising, since previous studies at Southwest Research Institute and elsewhere have encountered difficulties in the measurement of trace quantities of these compounds. With many available chromatographic

columns, both classes could not be measured because of adsorption or reaction on the column material.

To overcome this difficulty, various techniques were evaluated to prepare special columns that would perform satisfactorily in the separation and detection of micro amounts of amines and glycols.

Of the columns tested, the one that showed the most promise for measuring amines was 5 ft x 1/4 in. OD, 15 percent silicone DC 550 coated on a 12.5 percent KOH-treated 60/80 mesh Chromosorb P. This column was prepared by adding 12.5 percent of KOH, by weight, dissolved in ethanol to a known weight of Chromosorb P. The mixture was evaporated on a rotary evaporator to dryness. To this mixture, 15 percent (w/w) of DC 550 dissolved in acetone was then added, mixed well, and evaporated also to dryness. Further drying was accomplished by heating the mixture in an oven at 110° C for one hour. After the chromatographic column had been properly packed and installed in the instrument, it was necessary to "condition" it prior to use by passing nitrogen gas through it at 125 cc/min for a period of at least four hours. Proper packing of the coated solid support in the column is very important. Particles, for example, must be evenly distributed in the column so that there are no voids. This could be achieved by vibrating the chromatographic tube in order to distribute the coated support evenly.

A similar procedure was adopted for the preparation of a

new chromatographic column for separating and detecting micro amounts of glycols. The column that gave the best results was 5 ft x 1/4 in. OD, acid-washed 40/60 mesh G-Chromosorb solid support coated with 5 percent Reoplex 400 (polypropylene-glycol adipate). The processes of mixing and coating, evaporating and drying, packing and conditioning of the column were the same processes used in the preparation of the column used in the analysis of amines except that chloroform was used as a solvent.

Analysis of Sample Extracts. The concentrated extracts obtained from the spiked samples either by solvent extraction or micro distillation were analyzed by gas chromatography. The chromatograms obtained were compared with known calibration chromatograms of the same components that were originally added to the sample. The extract chromatograms from the spiked tissue sample and the chromatogram of the solvent, used in the extraction process, were also compared with the calibration chromatograms to determine if any of the components were present originally, in the sample or in the solvent.

In order to make a standard solution of an organic mixture, the components of the mixture and their respective densities should be known. Because the procedures for preparing such standards were almost the same for each homologous series investigated, only a description of preparing a standard solution of a blend of ketones is mentioned as an example. To make a 25-ml standard solution of this blend (con-

centration 1 µl = 10 nanograms per component), the following procedures were followed:

(a) Only pure chromatographic compounds and pure chromatographic solvents were used. The ketone blend used in this study was obtained from Poly Science Corporation, Evanston, Illinois. The solvent used, carbon tetrachloride, was obtained from Burdick and Jackson Laboratories, Inc., Muskegon, Michigan.

(b) A stock solution of the blend (concentration 1 µl = 1 µg for each component) was then prepared, calculated from the average density of the components of the mixture. In this case, the solution was prepared by dissolving 31.3 µl of the blend in 25 ml of carbon tetrachloride, based on an average density of 0.80 g/ml.

In using this solution, the tissue sample was spiked with 2.5 µg of each of the components of the blend. On a 5-g sample, this represented 0.5 ppm by weight for each component. This known amount of compound was dissolved in an organic solvent and was then carried through the extraction, cleanup, and analytical procedures. The final volume of the solution was reduced to 0.25 ml, so that 1 µl of this solution contained 10 nanograms of each compound investigated.

(c) The standard solution was then prepared from the stock solution. This was done by mixing 0.75 ml of the stock solution with 25 ml of carbon tetrachloride. The standard solution was prepared to give the same concentration as that of the extract; i.e., 1 µl = 10 nanograms of each compound investigated.

The peak area method was used as the standard method for quantitative analysis in the chromatographic study. It has been found that the weight concentration of a sample component is directly proportional to its peak area, compared to the peak area of the compound in a standard mixture of known composition tested under the same operating conditions.

To use this method, an aliquot of the concentrated extract was injected into the gas chromatograph and the chromatogram obtained. The relative recovery of each added compound in the extract was calculated from its peak area relative to that of the standard obtained by injecting the same volume as that of the extract into the gas chromatograph. Under the same operating conditions, the relative recovery of the extracted compound was computed from the peak areas produced by the standard and the final extract solution as follows:

$$\text{percent of recovery} = \frac{\text{peak area of compound} \times 100}{\text{peak area of standard}}$$

The peak area of a chromatogram was obtained by multiplying the peak height by the width of the peak taken at half height. This was done for sharp, symmetrical, and completely resolved peaks, such as for members of a homologous series.

Theoretical recovery was not obtained on any of the compounds investigated. In most cases, some losses were incurred during purification of sample extracts or during chromatography. Recoveries were relatively constant with all types of compounds tested, and the losses were thought to be due primarily to mechanical handling losses in sample processing.

Sensitivity is better with a flame ionization detector than with many other detectors that can be used. The methods developed in this study make it possible to detect as little as 2-4 nanograms of a compound as it emerged from the column. Thus, the ultimate limit of detection for most types of organic compounds tested was in the range of 0.1 ppm based on a 5-g sample, and quantitative results could be obtained on samples containing 0.3-0.5 ppm or more.

One problem that may be encountered in trace analysis using extraction with an organic solvent is that of interference from

solvent remaining in the final sample. To illustrate this problem and show typical chromatograms, a series of chart records is presented from laboratory experiments using oyster samples spiked with a number of aliphatic hydrocarbons. Pure compounds ranging from C_8 (n-hexane) to C_{18} (n-hexadecane) were used, and are indicated on the charts by the number of carbon atoms. Figure 4 shows a chromatogram obtained with the carbon tetrachloride solvent; the abrupt changes in the recording are due to changes in attenuation scale as noted on the chart (4X, 8X, etc.). The "tailing" effect shown is typical of many columns when relatively large amounts of carbon tetrachloride are injected into the instrument. In such a situation, trace components that are recorded before the beginning of the CCl_4 peak are not affected, those recorded during the "tailing" must be measured by measuring the peak area above the solvent background, and any that appear at or near the maximum point of the solvent peak may not be capable of measurement.

To illustrate this, Figure 5 shows the chart record obtained in a calibration run with carbon tetrachloride containing small amounts of nine hydrocarbons. Again, abrupt changes in the chart record are due to changes in attenuation scale. Despite the

"tailing" effect, however, identifiable peaks can be observed for each of the nine trace hydrocarbons in the sample.

Figure 6 shows a similar chart record obtained in analyzing an oyster sample which had been spiked with the same hydrocarbons. The C_8 and C_{12} hydrocarbons were used at a level of one part per million each, while the remaining hydrocarbons shown were used at a level of 0.5 ppm. The baseline is less stable than in Figure 5, due to minor interference from various constituents not completely removed in the sample preparation. However, separate peaks can be identified for each hydrocarbon and a quantitative or semi-quantitative estimate can be made of the amount present.

Calibration With Various Organic Compounds

With the availability of analytical techniques and satisfactory chromatographic methods, tests were conducted with a large number of spiked samples to obtain calibration data and confirm the validity of the entire procedure with different classes of compounds. A table listing pertinent data is included in the Appendix, and brief comments concerning the columns used and other experimental details are also included.

These results show that all of the various processing steps described previously can be used for sample processing and analysis, and that quantitative results can be obtained with many different types of organic compounds. The combination of processing steps that proved to be most useful included the following operations:

1. Grinding and treatment with sodium sulfate to avoid problems in subsequent extraction due to the presence of water.
2. Extraction with carbon tetrachloride.
3. Treatment by column chromatography to separate naturally occurring interfering substances.
4. Analysis by gas chromatography.

Figure 7 shows a flow diagram illustrating the various steps in processing samples by the preferred method, which was used in subsequent experiments.

Fish Exposure Studies

To confirm the utility of the methods developed, a series of laboratory tests was conducted in which fish were exposed to various organic compounds and the accumulated contaminants were measured. This made it possible to measure the absorption of contaminants during various periods of exposure, and to determine the recovery of contaminants and the sensitivity of the method with actual exposed samples as

a check on the validity of the experiments conducted with spiked samples.

Small fish were exposed in the laboratory in water containing known concentrations of contaminants for various periods of time. Samples were then analyzed and the results were compared with tests using spiked samples and analytical controls.

In the case of the long-term effects of the contaminants and their detection in fish, toluene and diethylene glycol of different concentrations were used. O-cresol was used as a contaminant, in one concentration only, for a short period of time.

Exposure Conditions. The type of fish used for the study was sheepshead minnow, and the average weight of each fish was two grams. The source of the fish and the dilution water was Galveston Bay near Seabrook, Texas. Salinity of the water used was 16 ppt.

The exposure of the fish to the contaminants was conducted in rectangular glass jars 24 cm in width, 28 cm in length, and 38 cm in height with a volume of 20 liters of bay water. A control jar and a minimum of four different concentrations were used for toluene and diethylene glycol.

Separate tests were made for each concentration of the contaminants by placing ten fish in each test jar containing 20 liters of bay water and observing the fish over a 96-hour period for toluene and diethylene glycol. This was done to maintain the recommended 2-g fish weight per liter of water. The toluene dilution series contained 20, 40,

80, and 160 mg/l, respectively. The diethylene glycol dilution series contained 8, 16, 32, and 64 g/l, respectively. In the case of the o-cresol test, the water used in the test contained only one concentration, 16 mg/l. Relatively high concentrations were chosen deliberately, to provide fish samples for laboratory use containing substantial amounts of contaminant. This would simulate a condition in a bay or estuary involving a substantial waste discharge that might endanger marine life.

Dissolved oxygen was maintained at or near the saturation level in each test jar by aeration. A diaphragm-type air pump and carborundum diffusers were used in the aeration system.

Dead fish were removed from the various test jars as soon as death occurred to prevent further contamination of the test environment by decaying fish. In the case of the toluene and diethylene glycol tests, the fish survivors were taken, cleaned up externally by rinsing them thoroughly with tap water, and then processed for the analytical procedures. However, in the o-cresol test, only dead fish were cleaned up and processed for analytical purposes because all the fish which were exposed to a concentration of 16 mg/l of this contaminant died within two hours.

Analytical Procedures. After exposure, fish samples were processed using the procedures described previously and illustrated in Figure 7. The resulting extracts were then analyzed by gas chromatography. The analytical procedures employed were as follows:

The column used for detecting toluene in the samples was a 12 ft x 1/8 in. Apiezon L packed column at a temperature of 110° C and a flow rate of about 70 cc/min. The solvent used for extraction was carbon tetrachloride.

The fish sample which was spiked with a known amount of toluene and the unspiked fish sample were run simultaneously for control purposes. The other fish samples, which were exposed to the different concentrations of the contaminant, were also run in order to determine the amount of toluene in each.

It was shown that a concentration in the range of 0.5 ppm of toluene could be measured in a 5-g prepared fish sample. At the end of the 96-hour exposure tests, the following results were obtained with various levels of exposure:

Exposure level, mg/l toluene in water	Measured level, ppm toluene in fish sample
20	6.7
40	11.6
80	17.0
160	20.5

In the analysis of diethylene glycol (DEG), a 5 ft x 1/4 in., five percent Reoplex column (on 40/60 mesh Chromosorb G) was used at a temperature of 200° C and flow rate of about 350 cc/min. The analytical procedure was similar to that used with toluene, except that

it was found that better recovery was obtained if fish samples were extracted with ethanol rather than with carbon tetrachloride as was done previously. The reason for this is not known, but may be related to chemical characteristics. Extraction of a polar compound such as DEG appears to be more successful with a polar solvent such as ethanol, whereas the relatively non-polar carbon tetrachloride had given best results in the past with non-polar constituents such as hydrocarbons.

A standard solution was used which contained 2 ppm DEG by weight, based on a 5-g sample of fish. This proved to be near the lower limit of detection, and thus DEG is more difficult to detect at lower concentrations than most other compounds tested previously. At higher concentrations, however, recovery of DEG from spiked samples was approximately 90 percent, in the same range with the better results obtained previously with other constituents.

At the end of 96 hours, analysis of the fish samples showed the following results:

Exposure level, mg/l DEG in water	Measured level, ppm DEG in fish sample
8	174
16	360
32	494
64	1630

The column used for detecting o-cresol in the samples was a 6 ft x 1/8 in., SE 30 column (on 60/80 mesh Chromosorb P) at a temperature of 180° C and a flow rate of approximately 70 cc/min. Carbon tetrachloride proved to be a satisfactory solvent for the extraction of o-cresol from fish samples. Concentrations as low as 0.5 ppm in fish could be detected.

Fish samples were exposed to only one concentration of o-cresol in bay water, 16 mg/l. At the end of two hours, all of the fish died due to exposure at this level. Analysis of fish samples showed a concentration of 0.62 ppm of o-cresol after this exposure, although this was near enough to the limit of detection that numerical accuracy may be only approximate.

Discussion of Results. Based on the results of these tests and other tests using spiked samples, some general statements can be made concerning the degree of recovery and the sensitivity of the methods of sample preparation and analysis that have been used.

The general extraction and cleanup procedure shown in Figure 7 was shown to be satisfactory for processing samples. Thus, detailed laboratory investigations utilizing a variety of marine samples could be conducted without using unduly complicated methods for processing a variety of samples. Different columns were used for the final analytical step in order to determine sensitivity and percent recovery under ideal conditions, but screening tests could be made to identify a

variety of contaminants (if present) in a single run.

Sensitivity and precision were similar with fish, shrimp, or oyster samples, and there is no reason to believe that other species would be substantially different in this respect. Based on comparisons of spiked and unspiked samples, together with solvent control tests and calibration chromatographs, the quantitative recovery of most constituents was in the range of 70 to 90 percent, with only few exceptions (see detailed data in Appendix). The lower limit of detection for most constituents is in the range of 0.2 to 1.0 ppm in marine specimens, again with only a few exceptions. Laboratory exposure tests with fish confirmed the accumulation of organic contaminants in the fish as a function of concentration in the water during exposure, and demonstrated the use of the methods developed in this study to make valid measurements.

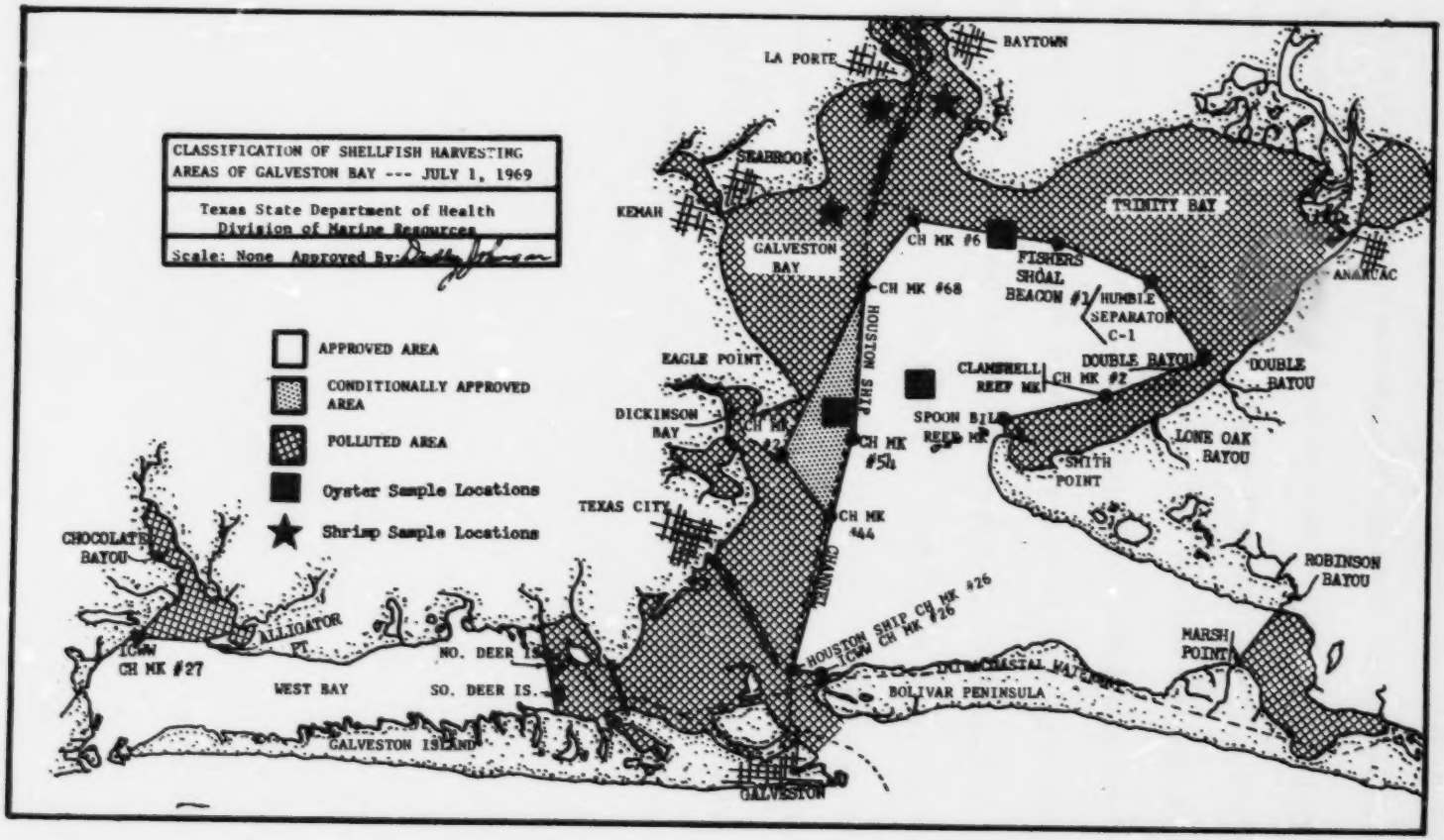
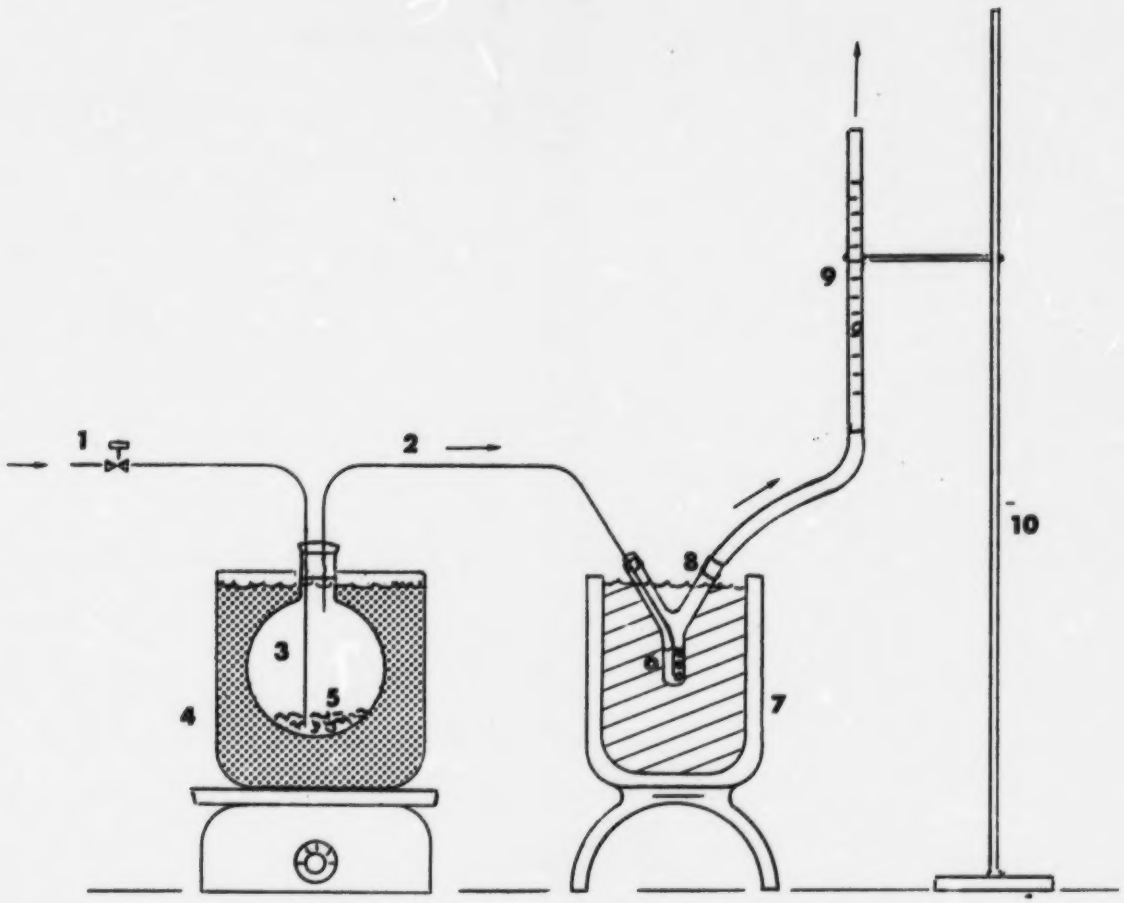


Figure 1. Locations of Marine Organisms Obtained From Galveston Bay



1. Nitrogen Flow 10 cc/min
2. Teflon Tubing (1 mm ID)
3. 125 ml Round Bottom Flask
4. Water Bath
5. Sample Slurry
6. Solvent Reservoir
7. Ice-Salt Bath
8. Microtrap
9. Rotameter
10. Stand

Figure 2. Micro Distillation Apparatus

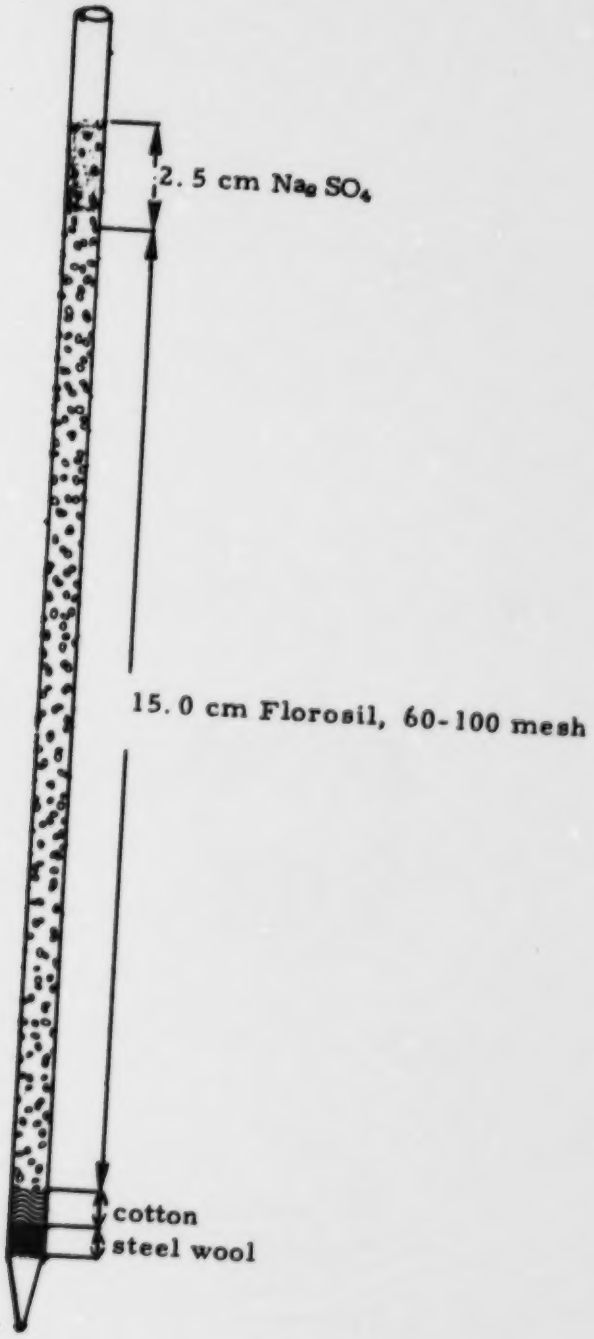


Figure 3. The "Cleanup" Column

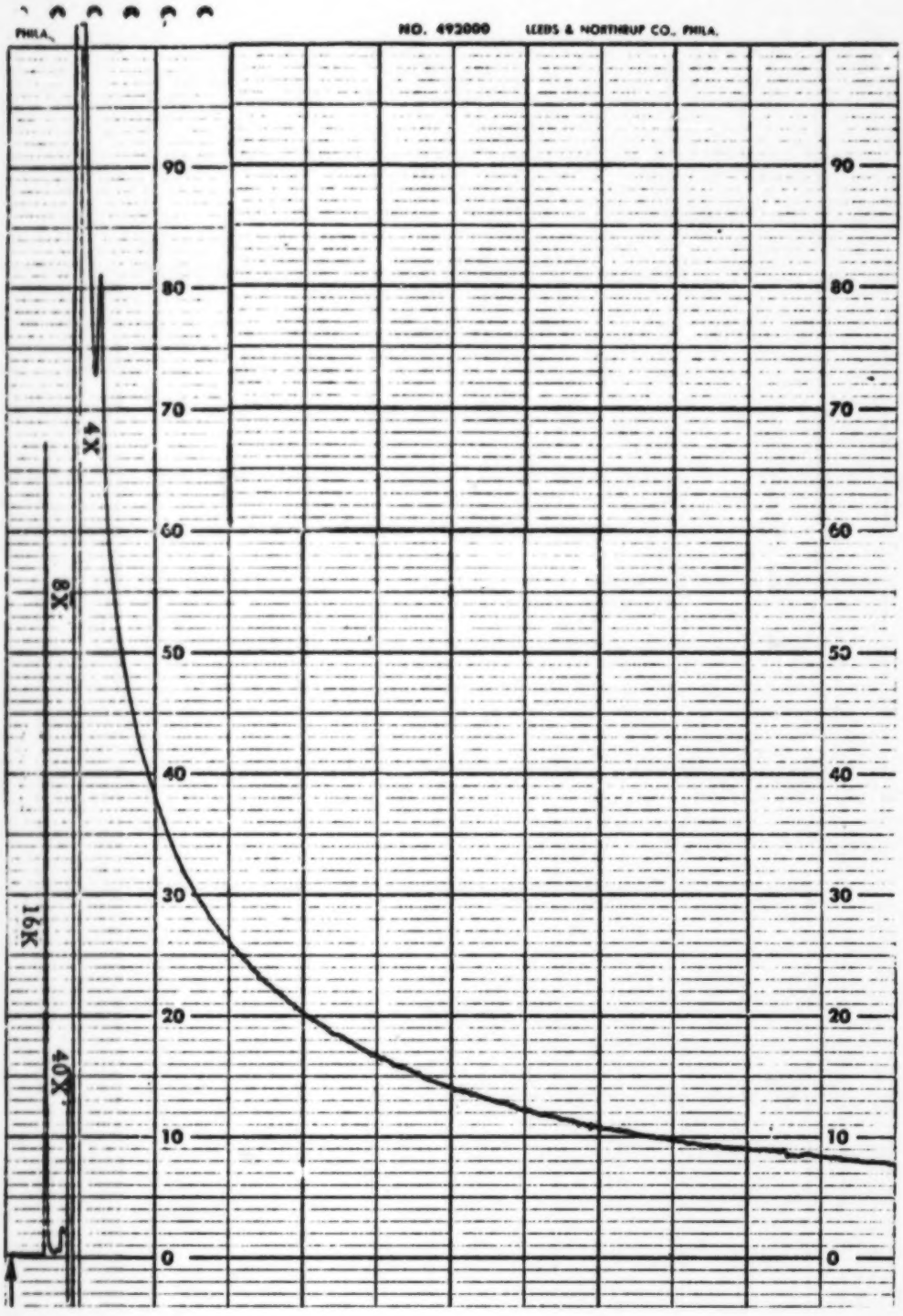


Figure 4. Chromatogram of Carbon Tetrachloride Solvent

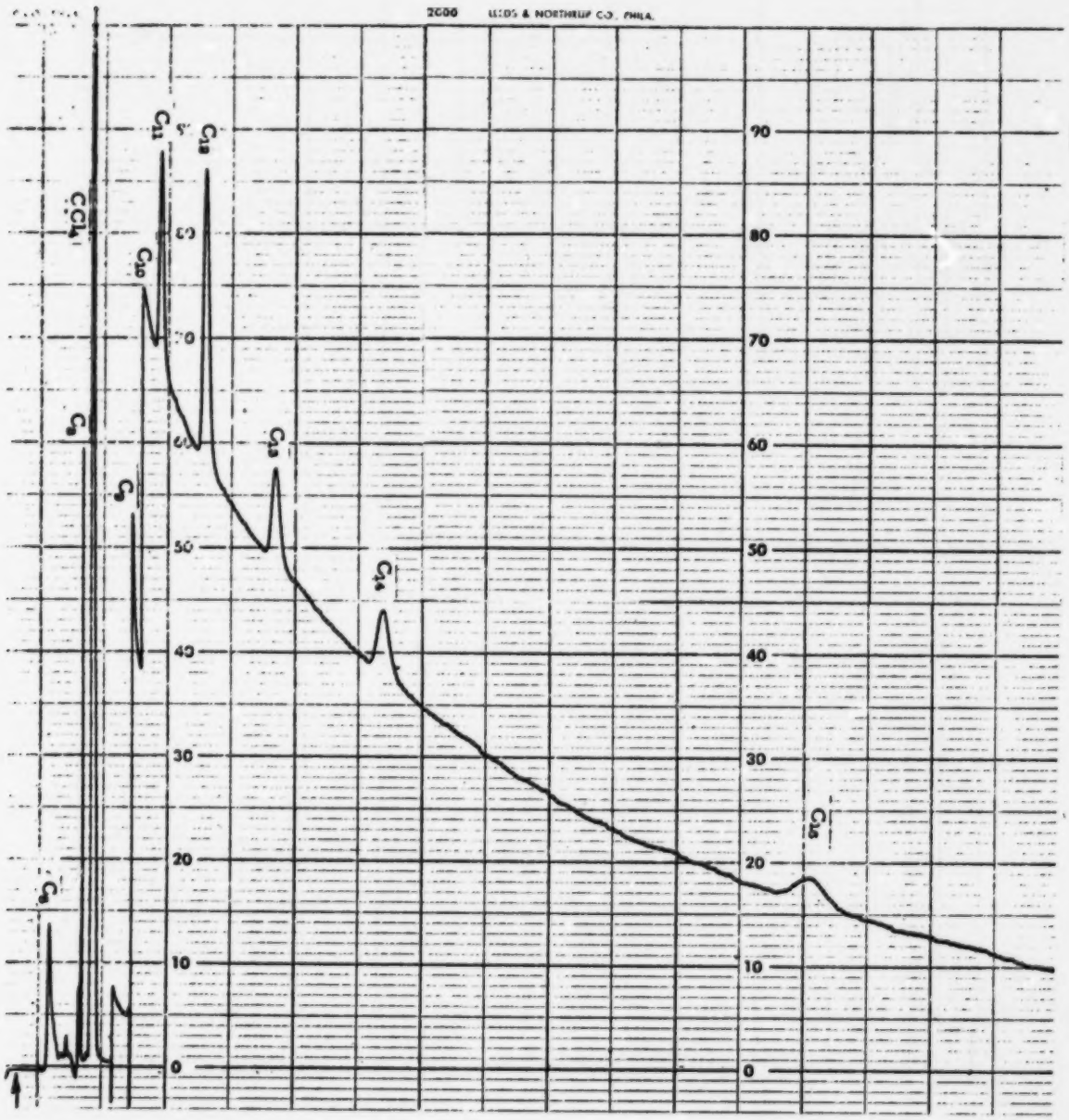


Figure 5. Chromatogram of Calibration Sample

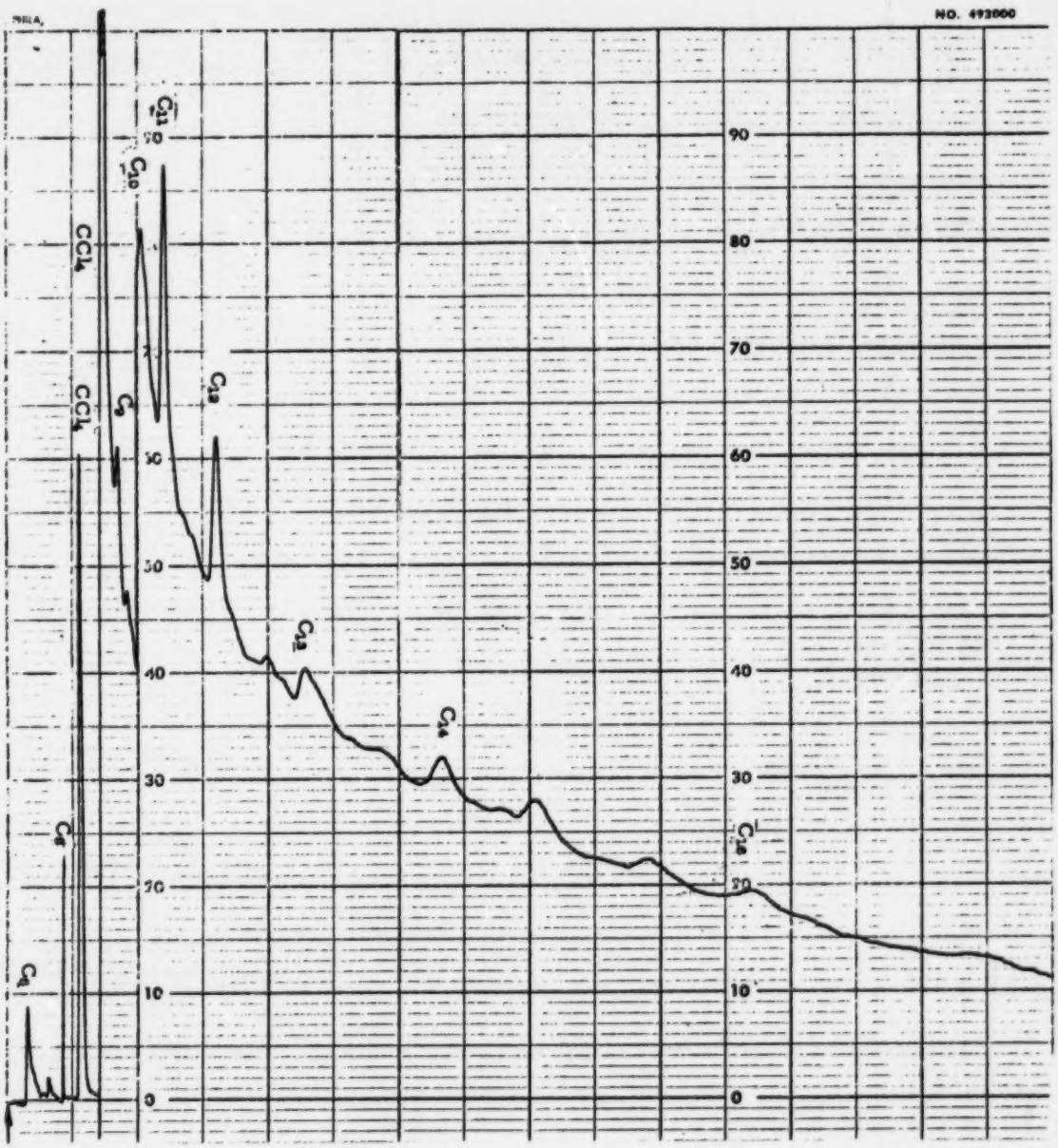
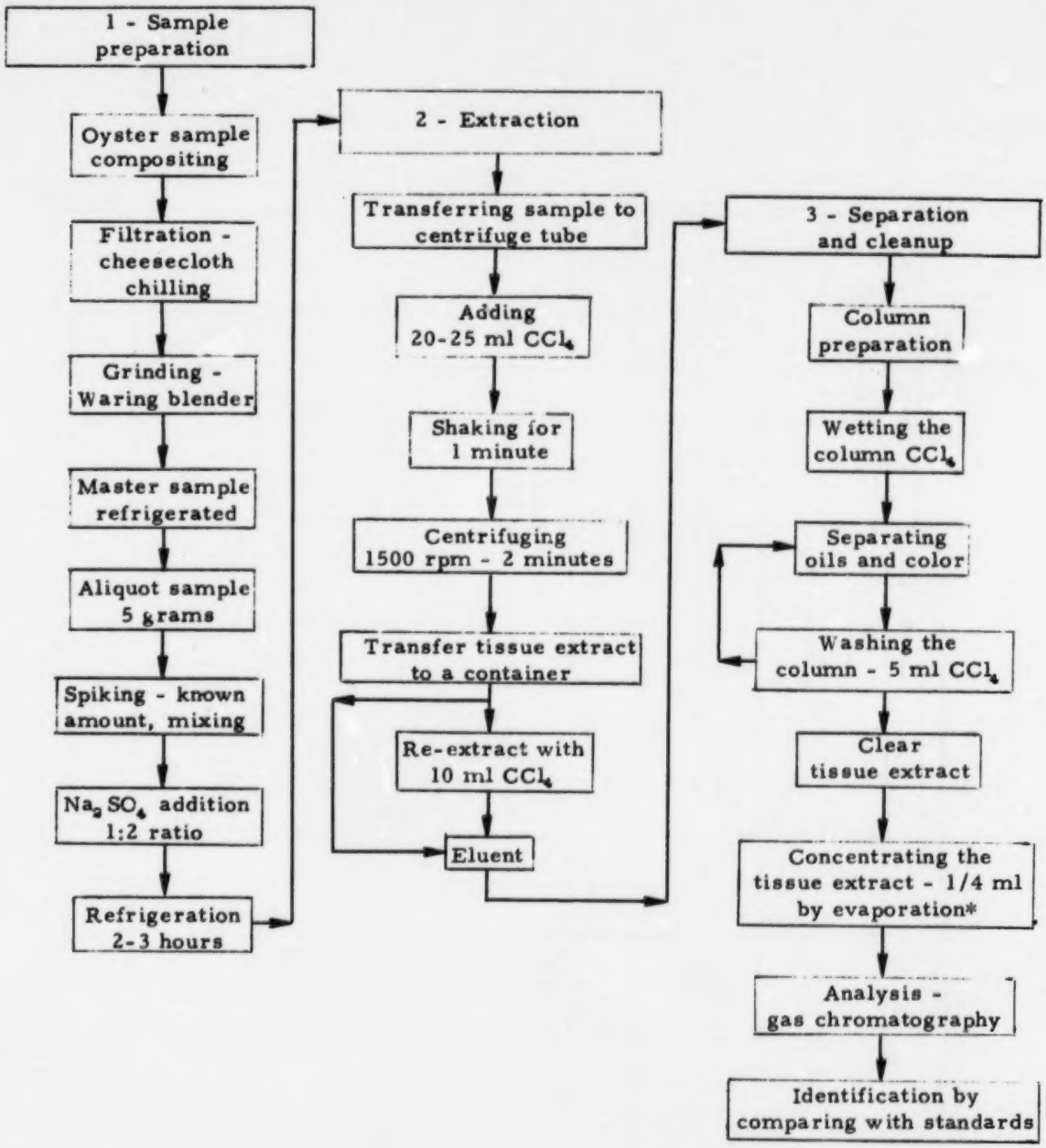


Figure 6. Chromatogram of Spiked Oyster Sample


1 - Sample preparation
Oyster sample compositing
Filtration - cheesecloth chilling
Grinding - Waring blender
Master sample refrigerated
Aliquot sample 5 grams
Spiking - known amount, mixing
Na_2SO_4 addition 1:2 ratio
Refrigeration 2-3 hours
2 - Extraction
Transferring sample to centrifuge tube
Adding 20-25 ml CCl_4
Shaking for 1 minute
Centrifuging 1500 rpm - 2 minutes
Transfer tissue extract to a container
Re-extract with 10 ml CCl_4
Eluent
3 - Separation and cleanup
Column preparation
Wetting the column CCl_4
Separating oils and color
Washing the column - 5 ml CCl_4
Clear tissue extract
Concentrating the tissue extract - 1/4 ml by evaporation*
Analysis - gas chromatography
Identification by comparing with standards

*Evaporation temperature depends on boiling points of contaminants investigated.

Figure 7. Sample Preparation Procedures

APPENDIX

This Appendix presents a discussion of the calibration tests that were performed with spiked samples, as described in the text of the report. Where pertinent, information is given on procedures, chromatographic columns used, results obtained, and other experimental details. A table is included, presenting detailed data on these calibration tests. Also, a Bibliography is included, listing literature references pertinent to the work described.

1. Aromatic Compounds

Extraction of the known aromatic compounds from spiked oyster samples was carried out by two methods, microdistillation and solvent extraction. The analysis of the concentrated extracts of each method differed and thus will be discussed separately.

Micro Distillation Extract. The concentrate which contained 0.25 ml of CCl_4 and the aromatic distillate was analyzed by gas chromatography using a column 12 ft x 1/8 in. OD, 10 percent Apiezon on Chromosorb P, 60/80 mesh. The separation was carried out at 120° C. The sample chromatogram, when compared to a calibration blend chromatogram, showed a high percentage of recovery.

Organic Solvent Extraction. The solvents considered for the extraction of aromatic compounds through C_9 from oyster samples were carbon tetrachloride, n-hexane, and n-dodecane. Though all of these solvents proved to be excellent solvents by preliminary extraction experiments of prepared water-aromatic standards, it was decided to investigate only n-hexane and n-dodecane. Samples spiked with known amounts of aromatic compounds were run simultaneously with the unspiked samples for control purposes. It was shown by analyzing the spiked samples that concentrations in the range of 0.1 ppm aromatics through C_9 could be detected in a 5-g prepared sample.

The extraction procedure using n-dodecane as a solvent was

identical to n-hexane extraction. As an extraction solvent, n-dodecane offered definite analytical advantages over n-hexane since it elutes after the aromatic compounds to C_9 using a silicone DC 550 open tubular chromatographic column. The lower vapor pressure also made n-dodecane an excellent solvent since there is less danger that it will evaporate during the extraction procedures. The limitation in using n-dodecane as a solvent was its purity. It would be necessary to obtain a solvent of high purity in order to apply it successfully.

2. Unsaturated Hydrocarbons

Chromatograms were also obtained for unsaturated hydrocarbons including 2-pentene, 2-hexene, and 2-octene. The column selected to perform the analysis was also 6 ft x 1/8 in. OD, 20 percent SE 30 packed on Chromosorb P, 60/80 mesh, at a temperature of 100° C. In the series of experiments performed, the tissue sample was spiked with 2.5 μg of each of the components studied. This concentration was measured under the conditions mentioned above, for each of the unsaturated hydrocarbons tested.

3. Saturated Hydrocarbons

The chromatograms of this class of compounds were obtained by analyzing a mixture of C_8-C_{18} saturated hydrocarbons. In the analysis, a 100 ft x 0.02 in. OD, silicone DC 550 open tubular gas chromatographic column was used at a temperature of 180° C. Based on a comparison of the spiked oyster extract chromatograms and calibration chromato-

grams, the quantitative recoveries were between 75 and 80 percent. This indicated that the sample preparation, extraction, cleanup, and concentration methods developed were adequate to recover the compounds investigated and provided semiquantitative results.

4. Alcohols

In the analysis of alcohols, which included 1-butanol, 1-pentanol, 1-hexanol, and 1-heptanol, a 6 ft x 1/8 in. OD, 20 percent SE 30 packed on Chromosorb P, 60/80 mesh gas chromatographic column was used at a temperature of 125° C. The concentration of each compound was 0.5 ppm by weight.

5. Phenols

The column used for detecting phenols in the samples was 6 ft x 1/8 in. OD, 20 percent SE 30 packed on Chromosorb P, 60/80 mesh, at a temperature of 180° C.

Oyster and shrimp samples were spiked with known amounts of phenols, including phenol, m-cresol, o-ethyl phenol, and p-ethyl phenol. These samples were also run simultaneously with unspiked samples for control purposes. It was shown by analyzing the spiked samples that concentrations in the range of 0.2 ppm of o-ethyl phenol could be measured in a 5-g prepared sample.

6. Ketones

The investigation of the oyster and shrimp samples studied had shown that some ketones, including 2-butanone, 2-pentanone, and

2,4-dimethyl-3-pentanone could be detected in the 0.5-ppm range in spiked samples. The chromatographic column used in the analysis was a 50 ft x 0.02 in. ID, silicone DC 550 column at a temperature of 180° C or 100° C. Chromatograms of some sample extracts showed some unknown peaks, and no attempt was made to identify them.

7. Amines

It was mentioned previously that amines could not be measured with many known chromatographic columns because of absorption or reaction on the column material. This difficulty was overcome by using a column that was 5 ft x 1/4 in. OD, 15 percent silicone DC 550 coated on a 12.5 percent KOH-treated 60/80 mesh Chromosorb P at a temperature of 120° C. Results using this column indicated that micro quantities of diethylamine, di-n-propylamine, di-n-butylamine, and cyclohexylamine could be measured in a manner similar to that used for other types of organic compounds, and accuracy and sensitivity were similar. Therefore, measurement of these amines to determine pollution by this class of organic compounds seemed to be assured. Spiked oyster and shrimp samples were used in testing for these amines, which constitute an important class of organic pollutants.

8. Glycols

Glycols had also been a very troublesome class of organic compounds investigated, from the standpoint of analysis by gas chromatography. Since they constitute an important class of organic pollutants,

it was felt that some method of measuring them would be desirable. Various available columns were evaluated to select one that would perform satisfactorily in detecting them, but none was good enough. The one column that showed the most promise was developed at Southwest Research Institute, and it is described fully under Section IV, "Preparation of New Chromatographic Columns". The column was 5 ft x 1/4 in. OD, 5 percent Reoplex 400 coated on acid-washed G-Chromosorb, 40/60 mesh. Results using it at a temperature of 180° -200° C indicated that micro quantities of glycols in the range of 1.0 ppm could be measured in both oyster and shrimp samples, in about the same range of sensitivity obtained in measuring other organic compounds.

Many solvents were used for extracting glycols from the spiked samples. The two that showed the best results were ethyl alcohol and carbon tetrachloride.

9. Ester – Methyl Hexanoate $C_5 H_{11} COOCH_3$

The column used for detecting methyl hexanoate in the samples was a 1-m x 2.3-mm Porapak Q, 80/100 mesh packed column at a temperature of 220° C and flow rate of 125 cc/min. The solvent used for extraction was carbon tetrachloride.

It was shown that a concentration in the range of 0.5 ppm of methyl hexanoate could be measured in 5-g prepared shrimp and oyster samples. The percent recovery of the compound in both samples was approximately 88 percent.

10. Heterocyclic Compound – Pyridine C_5H_5N

The column selected to detect pyridine in the samples was also a 1-m x 2.3-mm Porapak Q, 80/100 mesh packed column at a temperature of 175° C and flow rate of 175 cc/min. The solvent used for extraction was ethanol. Concentrations as low as 2.0 ppm in the samples could be detected. The percent of recovery in the shrimp sample was 50 percent whereas in the oyster sample recovery was 89 percent.

11. Butyric Acid – C_3H_7COOH

The column used for detecting butyric acid in the samples was a 5-ft x 1/4-in. acid-washed, 40/60 mesh G-Chromosorb solid support coated with 5 percent of Reoplex 400 at a temperature of 150° C and flow rate of approximately 200 cc/min.

Ethanol proved to be a satisfactory solvent for the extraction of butyric acid from the samples. Concentrations as low as 2.0 ppm in the shrimp and oyster samples could be detected.

The percent of recovery in the shrimp sample was approximately 67 percent whereas in the oyster sample recovery was 83 percent.

12. Diethyl Sulfide $(C_2H_5)_2S$

In the analysis of ethyl sulfide, a 6-ft x 1/8-in. SE 30, on 60/80 mesh Chromosorb P, column was used at a temperature of 70° C and flow rate of about 50 cc/min. It was found that better recovery was obtained if the shrimp and oyster samples were extracted with ethanol

rather than with carbon tetrachloride as was done in many cases previously. The reason for this is not known, but may be related to chemical and physical characteristics of the solvent.

A standard solution was used which contained 6 ppm of ethyl sulfide by weight, based on a 5-g sample of the organism. This proved to be near the lower limit of detection, and thus ethyl sulfide is again more difficult to detect at lower concentrations than most other compounds tested previously.

Recovery of ethyl sulfide from the spiked shrimp sample was approximately 78 percent, and recovery from the spiked oyster sample was 83 percent.

13. High Molecular Weight Hydrocarbon — N-Docosane
N-$C_{22}H_{46}$

The column used for detecting n-docosane in the samples was a 50-ft x 0.02-in. support-coated open tubular column packed with Carbowax 20M at a temperature of 175° C and flow rate of about 50 cc/min. The analytical procedure was similar to that used with other compounds, except that it was found that better recovery was obtained if the samples were extracted with ethyl benzene rather than with carbon tetrachloride or with ethanol as was done previously.

Two standard solutions were used which contained 0.5 ppm and 1.5 ppm of n-docosane by weight, respectively, based on a 5-g sample of shrimp or oyster. The lower concentration standard was used

to determine quantitatively the percent of recovery in the spiked samples. Recovery of n-docosane from the spiked shrimp sample was 73 percent whereas recovery from the spiked oyster sample was 88 percent.

14. Amide – N, N-Dimethyl Formamide $HCON(CH_3)_2$

The column used for detecting n, n-dimethyl formamide was a 1-m x 2.3-mm Porapak Q, 80/100 mesh packed column at a temperature of 180° C and flow rate of 60 cc/min. The solvent used for extraction was ethyl alcohol.

It was shown that a concentration of 1.0 ppm of n, n-dimethyl formamide could be measured in 5-g prepared shrimp and oyster samples. The percent recovery of the compound in the oyster sample was 90 percent and in the shrimp sample was 100 percent.

15. Chlorinated Hydrocarbon – Trichloroethylene C_2HCl_3

In the analysis of trichloroethylene, a 6-ft x 1/8-in. SE30 on 60/80 mesh Chromosorb P column was used at a temperature of 215° C and flow rate of 60 cc/min. It was found that good recovery was obtained when the shrimp and oyster samples were extracted with carbon tetrachloride as was done in many cases previously. Spiked samples were used which contained 2 ppm of trichloroethylene by weight based on a 5-g sample of the organism.

Recovery of trichloroethylene from the spiked shrimp sample was 90 percent, and recovery from the spiked oyster sample was also 90 percent.

16. Organophosphorus Compound – Tri-Iso-Propyl Phosphate $[(CH_3)_2 CHO]_3 P$

Only oyster sample was used in detecting tri-iso-propyl phosphate. The column used for detecting this compound in the sample was a 6-ft x 1/8-in. OD 15 percent PEG 20 M on Chromopak at a temperature of 150° C and flow rate of approximately 60 cc/min. The selection of the column came after trying different columns which were not satisfactory.

Different organophosphorus compounds were also used in other oyster samples, but their detection was not satisfactory. This class of organic compounds was extremely difficult to detect by means of gas chromatography under the previously described conditions.

Ethanol proved to be a satisfactory solvent for the extraction of tri-iso-propyl phosphate from the sample. Concentration as low as 30 ppm in the oyster sample was detected.

The percent of recovery was approximately 86 percent.

TABLE I. SUMMARY DATA SHEET OF CHROMATOGRAPHIC ANALYSIS OF SAMPLES

Organic Compounds	Formula	Gas Chromatography: Column	Conditions: Column Temp. °C	Flow N_2 ml/min	Inj. Temp. °C	Man. Temp. °C	N_2 Pres. lb	H_2 Pres. lb	Air Pres. lb	Tissue Extract: Theoret. Concentration ppm of Comp. per 5-g sample: Oyster	Shrimp	Fish	Sample Size µl Injected: Oyster	Shrimp	Fish	Percent Recovery: Oyster	Shrimp	Fish
1. Aromatics																		
benzene	C_6H_6	12 ft x 1/8 in. OD 100% Apiezon L packed on Chromosorb P 60/80 mesh	110	70	200	200	30	30	40	1	--	--	--	--	--	--	--	--
toluene	$C_6H_5CH_3$									1	--	--	--	--	--	89	--	--
ethyl benzene	$C_6H_5C_2H_5$									1	--	--	1	--	--	68	--	--
methyl xylene	$C_6H_4(CH_3)_2$									1	--	--	--	--	--	69	--	--
para xylene	$C_6H_4(CH_3)_2$									1	--	--	--	--	--	66	--	--
ortho xylene	$C_6H_4(CH_3)_2$									1	--	--	--	--	--	40	--	--
cumene	$C_6H_5CH(CH_3)_2$																	
2. Unsaturated Hydrocarbons																		
2-pentene	$CH_3CH=CHC_2H_5$	6 ft x 1/8 in. OD 20% SE 30 packed on Chromosorb P 60/80 mesh	100	50	210	200	20	30	35	0.5	0.5	--	--	--	--	67	47	--
2-hexene	$CH_3CH=CH(CH_2)_2CH_3$									0.5	0.5	--	0.2	0.2	--	76	27	--
2-octene	$CH_3CH=CH(CH_2)_4CH_3$									0.5	0.5	--	--	--	--	85	67	--
3. Saturated Hydrocarbons																		
n-hexane	C_6H_{14}	100 ft x 0.02 in. ID silicone DC 550	180	60	250	230	26	30	40	0.5	0.5	--	--	--	--	93	--	--
n-heptane	C_7H_{16}									0.5	0.5	--	--	--	--	--	--	--
n-octane	C_8H_{18}									1.0	1.0	--	--	--	--	--	76	--
n-nonane	C_9H_{20}									0.5	0.5	--	0.2	0.2	--	--	76	--
n-decane	$C_{10}H_{22}$									0.5	0.5	--	--	--	--	46	--	--
n-undecane	$C_{11}H_{24}$									0.5	0.5	--	--	--	--	90	80	--
n-dodecane	$C_{12}H_{26}$									1.0	1.0	--	--	--	--	95	--	--
n-tridecane	$C_{13}H_{28}$									0.5	0.5	--	--	--	--	89	50	--
n-tetradecane	$C_{14}H_{30}$									0.5	0.5	--	--	--	--	96	89	--
n-hexadecane	$C_{16}H_{34}$									0.5	0.5	--	--	--	--	57	89	--
4. Alcohols																		
1-butanol	$CH_3(CH_2)_2CH_2OH$	6 ft x 1/8 in. OD 20% SE 30 packed on Chromosorb P 60/80 mesh	125	50	200	210	25	30	40	0.5	0.5	--	--	--	--	--	--	--
1-pentanol	$CH_3(CH_2)_3CH_2OH$									0.5	0.5	--	0.2	0.2	--	--	--	--
1-hexanol	$CH_3(CH_2)_4CH_2OH$									0.5	0.5	--	--	--	--	53	75	--
1-heptanol	$CH_3(CH_2)_5CH_2OH$									0.5	0.5	--	--	--	--	--	--	--
5. Phenols																		
phenol	C_6H_5OH	same as above	180	50	210	210	25	30	40	0.9	0.9	--	--	--	--	67	75	--
m-cresol	$CH_3C_6H_4OH$									0.6	0.6	--	0.2	0.2	--	86	86	--
o-ethyl phenol	$C_2H_5C_6H_4OH$									0.2	0.2	--	--	--	--	75	62	--
p-ethyl phenol	$C_2H_5C_6H_4OH$									1.4	1.4	--	--	--	--	83	44	--
6. Ketones																		
2-butanone	$CH_3COC_2H_5$	100 ft x 0.02 in. ID silicone DC 550	100	50	200	200	20	30	40	0.5	0.5	--	--	--	--	77	85	--
2-pentanone	$CH_3CO(CH_2)_2CH_3$									0.5	0.5	--	0.2	0.2	--	86	60	--
2,4-dimethyl-3-pentanone	$(CH_3)_2CHCOCH(CH_3)_2$									0.5	0.5	--	--	--	--	80	40	--
7. Amines																		
diethylamine	$(C_2H_5)_2NH$	5 ft x 1/4 in. OD 15% silicone DC 550 coated on 12.5% Chromosorb P, 60/80 mesh	120	70	200	210	32	40	50	0.5	0.5	--	--	--	--	80	60	--
di-n-propylamine	$(CH_3CH_2CH_2)_2NH$									0.5	0.5	--	0.5	0.5	--	--	--	--
di-n-butylamine	$(C_4H_9)_2NH$									0.5	0.5	--	--	--	--	87	--	--
cyclohexylamine	$C_6H_{11}NH_2$									0.5	0.5	--	--	--	--	83	58	--
8. Glycols																		
ethylene glycol	CH_2OHCH_2OH	5 ft x 1/4 in. OD 5% Reoplex 400 coated on acid-washed G-Chromosorb, 40/60 mesh	200	70	250	235	26	40	50	1.0	1.0	--	0.2	0.2	--	75	--	--
diethylene glycol	$HO(CH_2)_2O(CH_2)_2OH$									1.0	1.0	--	--	--	--	80	80	--

TABLE I. SUMMARY DATA SHEET OF CHROMATOGRAPHIC ANALYSIS OF SAMPLES
(Continued)

Organic Compounds	Formula	Gas Chromatography: Column	Conditions: Column Temp. °C	Flow N_2 ml/min	Inj. Temp. °C	Man. Temp. °C	N_2 Pres. lb	H_2 Pres. lb	Air Pres. lb	Tissue Extract: Theoret Concentration ppm of Comp. per 5-g sample: Oyster	Shrimp	Fish	Sample Size µl Injected: Oyster	Shrimp	Fish	Percent Recovery: Oyster	Shrimp	Fish
9. Ester methyl hexanoate	$CH_3(CH_2)_4CHHCH_3$	1 m x 2.3 mm, Porapak Q, 80/100 mesh	220	125	210	240	30	30	50	0.5	0.5	--	0.2	0.2	--	88	88	--
10. Heterocyclic Compound pyridine	C_5H_5N	1 m x 2.3 mm, Porapak Q, 80/100 mesh	175	125	210	200	30	30	50	2	2	--	0.5	0.5	--	89	50	--
11. Acid butyric acid	C_3H_7COOH	5ft x 1/4 in., 5% Reoplex on G Chromosorb, 40/60 mesh	150	200	210	200	30	30	50	2	2	--	0.5	0.5	--	83	67	--
12. Sulfide diethyl sulfide	$(C_2H_5)_2S$	6 ft x 1/8 in. OD 20% SE 30 packed on Chromosorb P, 60/80 mesh	70	50	210	200	30	30	50	8	8	--	0.3	0.3	--	83	78	--
13. High Mol. HC's n-docosane	$n\text{-}C_{22}H_{46}$	50 ft x 0.02 in., open tubular column packed with Carbowax 20 M	175	50	210	200	58	30	50	2	2	--	0.3	0.3	--	88	73	--
14. Amide n, n-dimethyl formamide	$HCON(CH_3)_2$	1 m x 2.3 mm, Porapak Q, 80/100 mesh	180	60	240	210	60	30	50	1	1	--	0.3	0.3	--	90	100	--
15. Chlorinated HC trichloroethylene (Fisher),	C_2HCl_3	6 ft x 1/8 in. OD 20% SE 30, packed on Chromosorb P, 60/80 mesh	60	60	215	210	30	30	50	2	2	--	0.3	0.3	--	90	90	--
16. Organophosphorus Compound (1) tri-iso-propyl phosphate	$[(CH_3)_2CHO]_3P$	6 ft x 1/8 in. OD, 15% PEG 20 M on Chromopak	150	60	250	235	90	34	50	30(A)	--	--	0.5	--	--	80	--	--

(1) Two standard solutions were used. The lower standard was 10 ppm. The higher concentration standard, 30 ppm, was used to determine quantitatively the percent of recovery in the spiked sample.

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